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Matyáš Smolík

Cholinergic signalling in the striatum and its significance in cognitive flexibility
Význam cholinergní signalizace ve striatu pro řízení kognitivní flexibility

Bachelor´s thesis

Supervisor: MUDr. Helena Janíčková, Ph.D.

Consultant: doc. RNDr. Jiří Novotný, DSc.

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Abstract

In the striatum, cholinergic interneurons (CINs) contribute to the control of behaviour, motor and cognitive functions. Recently, number of studies have shown a special significance of CINs in the control of cognitive flexibility: the ability to learn new behavioural strategies when requirements of the environment change. Along with working memory, cognitive inhibition, attention control and other cognitive domains, cognitive flexibility belongs to executive functions. Cognitive flexibility impairment is present in a range of neuropsychiatric disorders and thus, understanding its mechanisms is of outstanding importance. The proposed work will first describe anatomy and cellular composition of the striatum and its functions. It will further describe cholinergic system with a special attention to cholinergic signalling in the striatum. The final chapter of the general part of the thesis will focus on cognitive flexibility. After discussing the involved structures and systems separately, the thesis will eventually provide comprehensive review of currently available studies investigating how striatal CINs contribute to brain's ability to replace old concepts with new and more efficient ones.

Keywords: striatal cholinergic interneurons, acetylcholine, dorsomedial striatum, cognitive flexibility, behavioural tasks

Abstrakt

Cholinergní interneurony ve striatu se podílejí na řízení chování, motoriky a kognitivních funkcí. V poslední době celá řada publikací poukázala na jejich zvláštní význam pro kognitivní flexibilitu, tedy schopnost naučit se novým strategiím chování a přizpůsobovat se měnícím se podmínkám prostředí. Spolu s pracovní pamětí, kognitivní inhibicí, pozorností a jinými funkcemi patří kognitivní flexibilita mezi exekutivní funkce. Protože narušená kognitivní flexibilita je jedním ze symptomů celé řady neuropsychiatrických onemocnění, má studium jejích mechanismů prvořadý význam. Tato práce nejprve pojednává o anatomii striata, zastoupení jednotlivých typů neuronů ve striatu a o jeho funkcích. Poté se zaměří na popis cholinergního systému a na specifika cholinergní signalizace ve striatu. Třetí obecná část práce pojednává o kognitivní flexibilitě. Nakonec práce po obecném úvodu přinese podrobný přehled dostupných studií, které nám zatím částečně umožnily pochopit význam cholinergní signalizace ve striatu pro schopnost mozku potlačovat staré koncepty a nahrazovat je novými.

Klíčová slova: cholinergní interneurony striata, acetylcholin, dorsomediální striatum, kognitivní flexibilita, behaviorální testování

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Statement of authorship

I hereby declare that I am the sole author of this bachelor thesis and that I have not used any sources other than those listed in the bibliography and identified as references. I further declare that I have not submitted this thesis at any other institution in order to obtain a degree.

Place, Date

Signature

List of abbreviations

AHP – Afterhyperpolarization

ACh – Acetylcholine

AChE – acetylcholine esterase

cAMP – cyclic adenosine monophosphate

ChAt – Choline Acetyltransferase

AChR – acetylcholine receptor

CINs – Cholinergic interneurons

DA – dopamine

DLS – dorsolateral striatum

DMS – dorsomedial striatum

GABA – gamma-Aminobutyric acid

GPi – Globus pallidus internus

GPe – Globus pallidus externus

IP3 – inositol triphosphate

LTD – laterodorsal tegmental nucleus

mAChRs – muscarinic acetylcholine receptors

nAChRs – nicotinic acetylcholine receptors

NPY – neuropeptide Y

MSNs – medium spiny neurons

SNc – substantia nigra pars compacta

SOM – somatostatine

Pf – parafascicular nucleus

PPT – pedunculopontine nucleus

STN – subthalamic nucleus

TAN – tonically active neurons

VAChT – vesicular acetylcholine transporter

VTA - ventral tegmental area

VMS – ventromedial striatum

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1 Introduction

Cognitive flexibility is the skill essential for adaptations to and survival in ever changing environment. Neural circuits critical for the update of behaviour to changes in the environment consist of prefrontal cortex including the orbitofrontal, prelimbic and infralimbic cortical areas, basal ganglia circuits and thalamus (Bissonette et al., 2013; Block et al., 2007; Ragozzino et al., 2002a). Here we focus on the striatum, the main input nucleus of the basal ganglia, which is important in learning new strategies and especially in their adaptation.

Although neurochemical processes within the striatum involved in cognitive flexibility are not completely understood yet, one neurotransmitter that has been shown to play an important role in facilitating cognitive flexibility is acetylcholine (ACh) (Cutuli et al., 2009; Tait et al., 2013). Principal source of ACh in the striatum are intrinsic cholinergic interneurons (CINs). Because these interneurons show spontaneous pacemaker activity, they are also referred to as tonically active neurons. Their large somata and extensive axonal arborizations throughout the striatum distinguish them from the more abundant GABAergic projection neurons and other types of striatal interneurons. Their rich arborization together with their tonic firing activity are important features that enable them to affect striatal output (Bolam et al., 1984; Dautan et al., 2014).

Since the first study of (Apicella et al., 1991) that recorded the responsiveness of putative CINs to specific environmental stimuli in primates, a number of studies emerged investigating the involvement of CINs in the control of learning and cognitive flexibility. In this thesis, I will present the review of these studies together with basic anatomical and physiological descriptions of the striatal regions and CINs.

2 Basic anatomical features of the striatum

The striatum, or corpus striatum (also called the neostriatum or the striate nucleus) is a nucleus (a cluster of neurons) in the subcortical basal ganglia of the forebrain.

It is usually divided into ventral and dorsal striatum based on anatomical localization and distinct connectivity. In primates, the ventral striatum consists of the nucleus accumbens and the olfactory tubercle. The dorsal striatum consists of the caudate nucleus and the putamen. Capsula interna, white matter in the dorsal striatum, separates the caudate nucleus and the putamen. Anatomically, the term striatum refers to its striped appearance of grey and white matter (Alexander, 1986).

In rodents, the striatum is described as a single mass of grey matter often referred to as caudate-putamen complex. The ventral striatum consists of the nucleus accumbens and the striatal portion of the olfactory tubercle, along with the ventromedial extension of the caudate nucleus and putamen. The nucleus accumbens comprises a core and a shell subregion, which are two anatomically and functionally defined areas that are well described in rodents, but less in primates. In general, the rostral most, medial, lateral and ventral parts of the accumbens are referred to as the shell, while its dorsal and central portions constitute the core. The dorsal striatum in rodents is commonly divided into its dorsomedial and dorsolateral part (broadly corresponding to caudate and putamen in primates, respectively).

2.1 Patch (striosomes)/matrix system

Another level of striatal compartmentalization is the patch/matrix system, which is largely based on the heterogenous distribution of various neurochemical markers and differential afferent and efferent connections. Patches or striosomes have been defined immunohistochemically as expressing high levels of μ-opioid receptor (MOR), substance P (SP), dopamine (DA) 1-receptor (D1R), met-enkephalin (met-ENK), calretinin, Nuclear Receptor Subfamily 4 Group A Member 1(Nr4a1), pro-dynorphin, glutamate decarboxylase 2 (GAD-2), and early growth response 1(EGR-1). The extrastriosomal matrix by contrast is enriched with calbindin, somatostatin (SST), enkephalin (ENK), DA2-receptor (D2R) and cholinergic markers including acetylcholine esterase (AChE) and choline acetyltransferase (ChAT) (Crittenden and Graybiel, 2011). Although the functional significance of this patch/matrix compartmentalization has been poorly understood, evidence that these two

compartments are differentially affected in some basal ganglia disorders is of significant interest (Bolam et al., 1988; Crittenden and Graybiel, 2011; Haber, 2003; Holt et al., 1997).

3 Striatal connectivity

3.1 Striatal input

The striatum receives glutamatergic input from the cerebral cortex and thalamus, dopaminergic input from the substantia nigra pars compacta (SNc) (targeting dorsal striatum) and ventral tegmental area (VTA) (targeting ventral striatum) and serotonergic and noradrenergic inputs from the dorsal raphe and locus coeruleus of the brainstem, respectively (Smith and Bolam, 1990). In addition, brainstem cholinergic neurons send projections to the striatum, the more rostral pedunculo-pontine nucleus (PPT) mostly innervates the dorsolateral striatum, while the laterodorsal tegmental nucleus (LDT) mostly innervates the medial striatum and the core of the nucleus accumbens (Dautan et al., 2014).

The glutamatergic cortical input to the striatum is topographically and functionally organised. The dorsomedial striatum, which is involved in goal-directed motivated behaviours mostly, receives inputs from the associative parts of the cortex, ventral hippocampus, basolateral amygdala and prelimbic area of the prefrontal cortex. The dorsolateral striatum which is more involved in mediating motor activity, movement and cue-driven habitual responses receives inputs preferably from sensorimotor cortex (Guo et al., 2015; Hunnicutt et al., 2016).

Another possible approach to the classification of striatal cortical inputs is based on the patch/matrix system. The input to striosomes or patches primarily arises from the limbic cortex, specifically from the orbitofrontal cortex and insula while matrix input arises from the motor cortex, somatosensory area and parietal lobe (Flaherty and Graybiel, 1994; Gerfen, 1989; Ragsdale and Graybiel, 1991).

Glutamatergic projections from the thalamus primarily arise from the intralaminar nuclei (particularly the centromedian and parafascicular nucleus), from motor relay nuclei (the anterior ventral and ventral lateral nucleus), and from the posterior thalamus (the posterior lateral nucleus and pulvinar) (Kato et al., 2011).

Nigrostriatal projections innervate both striosomes and matrix. They constitute one of the four major dopamine pathways in the brain, and the nigrostriatal pathway is particularly involved in the production of movement, as part of a system called the basal ganglia motor loop

(Tritsch et al., 2012). In addition, dopamine projections from the VTA to the ventral striatum are crucial for the development and control of reward-based behaviour.

3.2 Striatal output

Striatal projection neurons transmit signals via two main different routes to output nuclei, referred to as direct and indirect pathway (Figure 1). The direct pathway also called as GO pathway is formed by striatal projection neurons that preferentially express dopamine D1 receptor. This pathway projects directly to the output nuclei globus pallidus internus (GPi) and substantia nigra pars reticulata. In contrast, the indirect pathway or the NO-GO pathway formed by D2R-expressing striatal projection neurons projects to the output nuclei via the external segment of globus pallidus (GPe) and the subthalamic nucleus (STN) (Albin et al., 1989).

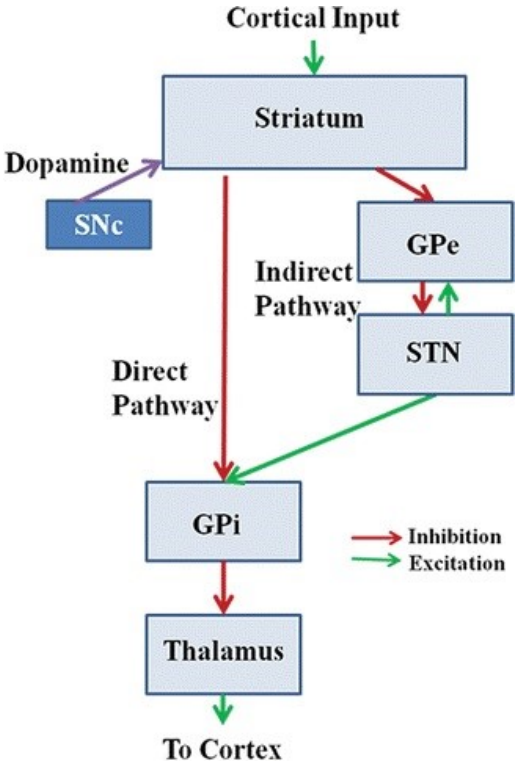

Figure 1: Direct and indirect pathways of basal ganglia, adopted from Chakravarthy 2015

Striatal projection neurons are GABAergic; therefore, the excitation of these neurons by cortical input leads to temporary inhibition of the GPi, which GABAergic neurons and their spontaneous activity inhibits thalamus. Thus, the activation of the direct pathway leads to inhibitions of GPi neurons, which in turn leads to disinhibition of the thalamus. Thalamus then can activate (with glutamate) its target cortical regions, which leads to increase in motor

activity. On the contrary, when the indirect pathway is activated, the thalamus is inhibited because GABAergic neurons from the GPe inhibit the STN and the projection neurons from the STN to GPi are glutamatergic. Hence, the GPi is activated and it inhibits the thalamus and the activation of the indirect pathway causes inhibition of motor activity (Nambu et al., 2002).

3.3 Functions of the striatum

Functionally, the striatum coordinates multiple aspects of cognition, including both movement and action planning, decision-making, motivation, reinforcement, and reward perception. The striatum is a critical component of the motor and reward systems; receives glutamatergic and dopaminergic inputs from different sources; and serves as the primary input to the rest of the basal ganglia.

According to their distinct functions in rodents, striatum can be divided into four parts. The dorsal striatum is involved in sensorimotor functions as serial order learning (Yin, 2010), stimulus-response habit formation (Devan et al., 2011) and performance of learned instrumental tasks (Shiflett et al., 2010). On the other hand, ventral striatum is involved in reinforcement of appetitive behaviours including drugs of abuse and natural rewards, such as food intake (Robinson and Berridge, 2000). Moreover, there is a functional difference between the lateral and medial part of the dorsal striatum. Lateral portion receives strong input from motor and premotor cortex thus is especially involved in motor learning and habit formation (Haber et al., 2000; Künzle, 1975). The dorsomedial striatum receives inputs from limbic regions, associative and prefrontal cortex and is involved in behavioural flexibility, reward associated motor learning and reaction time (Ragozzino, 2003). Recent studies have shown that the most posterior part of striatum, so called tail of the striatum, represents the fourth functional subdivision. Tail of the striatum receives strong inputs from the auditory, visual, and rhinal cortices, as well as from the amygdala, suggesting that this area may process multi-modality sensory inputs in the context of emotional information (Hunnicutt et al., 2016).

3.4 Cellular composition of the striatum

Striatum is a heterogeneous structure composed of mixture of different cell types. The most common neurons in the striatum are GABAergic striatal projection neurons, also called medium spiny neurons (MSNs), which are the principal output cell type. The MSNs, which preferentially express dopamine D1 receptors project to globus pallidus internus and substantia nigra pars reticulata. This projection is known as direct pathway or the GO pathway. The second group of MSNs, which preferentially express the D2 receptors project to the globus pallidus

externus (GPe), this tract is referred as indirect pathway or NO-GO pathway. Approximately 6% of MSNs in the dorsal striatum express both D1 and D2 receptors. These cells produce GABA and glutamate, allowing them to modulate the basal ganglia connections from both directions (Perreault et al., 2012).

Other cell types include interneurons. Approximately 4 % of striatal neurons are GABAergic interneurons. The classification of GABAergic interneurons is still incompletely understood, however, there are several subtypes identified so far. The currently recognized types of striatal interneurons include parvalbumin-expressing (PV+) fast spiking interneurons (FSIs), NPY (neuropeptide Y)/SOM (somatostatine)/NOS (nitric oxide synthase) expressing interneurons also referred to as low threshold spiking interneurons (LTSIs), NPY-expressing neurogliaform interneurons, calretinin-expressing low threshold spiking interneurons, tyrosine hydroxylase-expressing (TH+) interneurons with heterogeneous firing patterns and fast adapting interneurons (Tepper et al., 2018). The different types of GABAergic interneurons in the striatum have been classified based on electrophysiological properties, the expression of various neurochemical markers and/or synaptic connectivity.

The remaining 1% of striatal neurons is represented by cholinergic interneurons (CINs). Despite their relatively low number, they exert powerful modulatory effect over the entire striatum due to their tonic firing activity and dense axonal arborization. While there are also cholinergic projections coming from the brainstem cholinergic nuclei, the CINs are considered as the main source of striatal acetylcholine (Bolam et al., 1984; Dautan et al., 2014).

4 Cholinergic system and signalling

Cholinergic system provides diffuse connections to variety of brain areas. It is composed of nerve cells that synthetize and use ACh as a neurotransmitter for transduction of action potentials. ACh is widely distributed in nervous system, in mammalian brain represents one of the main neurotransmitters used by several major cell groups and pathways.

4.1 Localization of cholinergic neurons

Based on immunohistochemical studies detecting ChAT-expressing neurons, there are eight major groups of cholinergic neurons: Ch1 – Ch8. Ch1-4 are located in the basal forebrain. Ch1 is associated with the medial septal nucleus, Ch2 is associated with vertical nucleus

of diagonal band of Broca, Ch3 with the horizontal limb of diagonal band of Broca and Ch4 with the nucleus basalis of the substantia innominata. Ch5 and Ch6 are located in two closely related brainstem nuclei: Ch5 with the PPT nucleus of the rostral brain stem, Ch6 with the LDT nucleus of the rostral brain stem. Ch7 is associated with the medial habenula and Ch8 is associated with the neurons in the parabigeminal nucleus (Mesulam and Geula, 1988).

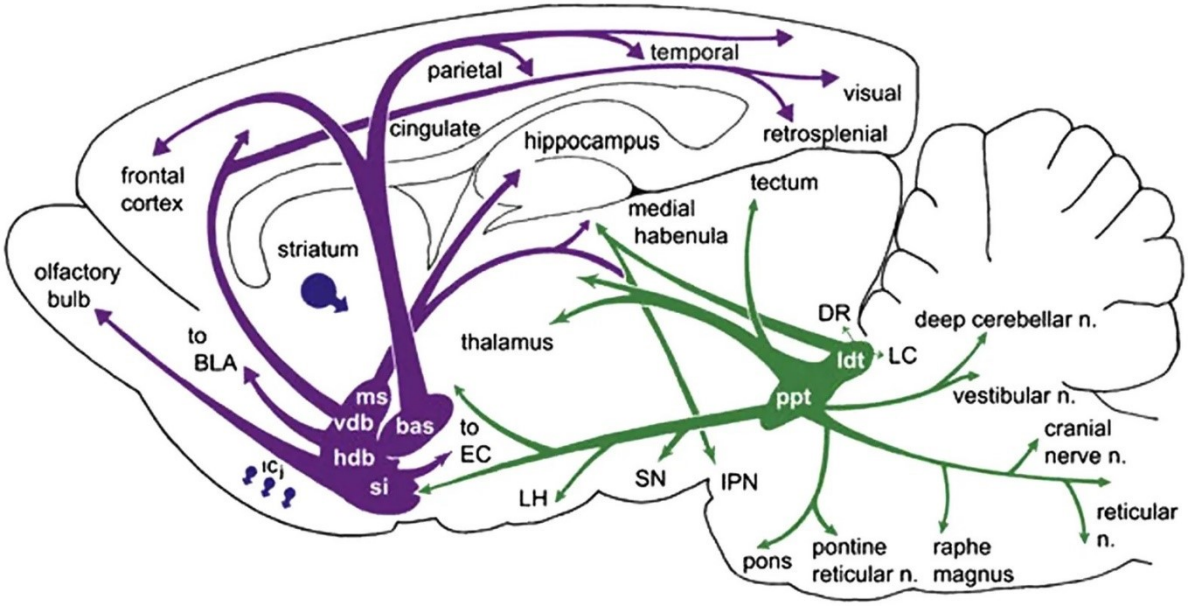

Figure 2: Cholinergic system in rodent brain.

bas, nucleus basalis; BLA, basolateral amygdala; DR, dorsal raphe; EC, entorhinal cortex; hdb, horizontal diagonal band nucleus; Icj, islands of Cajella; IPN, interpeduncular nucleus; LC; locus ceruleus; ldt, laterodorsal tegmental nucleus; LH, lateral hypothalamus; ms, medial septal nucleus; PPN, pedunculopontine nucleus; si, substantia innominata; SN, substantia nigra; vdb, vertical diagonal band nucleus. Adopted from Perez-Lloret 2016

4.2 ACh synthesis and turnover

ACh is synthetized from acetyl coenzyme A and choline by the enzyme choline acetyltransferase. Coenzyme A is synthesized in mitochondria and accesses choline acetyltransferase following transport across the mitochondrial membrane into the cytoplasm. Most of the choline used in ACh synthesis is thought to come directly from recycling of released ACh, hydrolysed to choline by acetylcholinesterase (AChE) in the synaptic cleft (Wessler et al., 1999). Choline transport through plasmatic membrane is mediated by high affinity choline transporter (CHT1). Another source of choline is the breakdown of phosphatidylcholine, which may be stimulated by locally released ACh (Fujii et al., 2009). After synthesis, ACh is loaded into synaptic vesicles by vesicular acetylcholine transporter (VAChT). ACh release into the

synaptic cleft requires the presence of Ca2+ ionts which enter the neuron during depolarization (Dodge and Rahamimoff, 1967).

4.3 ACh receptors

There are two types of receptors that bind ACh and transmit its signal, muscarinic AChRs and nicotinic AChRs (mAChRs and nAChRs), which are named after the agonists muscarine and nicotine, respectively. The main difference between these two types is that the muscarinic receptors are G-protein-coupled receptors that mediate metabolic response via second messengers such as cAMP, while nicotinic receptors are ligand-gated ion receptors.

4.3.1 Muscarinic receptors

Muscarinic receptors are selectively activated by the alkaloid muscarine from the mushroom *Amanita muscaria* and are blocked by belladonna alkaloids, such as atropine and scopolamine. The mAChRs are expressed in the CNS and also in other organs. They are part of a large family of G-protein-coupled receptors (GPCRs), which use an intracellular second messenger system to affect ion channels (Rosenbaum et al., 2009).

There are five subtypes of mAChRs differing in their structure, expression pattern and pharmacological activity: M1-M5. All five are found in the CNS, while M1-M4 are also found in other tissues: M1 mAChRs are common in secretory glands (Tobin et al., 2002); M2 mAChRs are found in cardiac tissue (Tietje and Nathansons, 1991); M3 mAChRs are found in smooth muscles and in secretion glands. M1, M3 and M5 receptors are coupled with Gq proteins which activation leads to the activation of phospholipase C, generating two secondary messengers (IP3 and DAG) eventually leading to an intracellular increase of calcium. The M2 and M4 subtypes are coupled with inhibitory Gi proteins and their activation leads to the inhibition of adenylate cyclase, thereby decreasing the production of the second messenger cAMP. The activation of the M2 receptor in the heart is important for closing calcium channels to reduce the force and rate of contraction (Caulfield, 1993).

4.3.2 Nicotinic cholinergic receptors

The nAChRs are ligand-gated ion channels mediating fast signal transmission at synapses. Nicotinic AChRs are involved in a wide range of physiological processes and can be either neuronal or muscle-type. Muscle-type nAChRs are localised at neuromuscular junctions. In addition, several distinct types of neuronal nAChRs are expressed in the CNS

where they are involved in cognitive functions, learning and memory, arousal, reward-related behaviour, motor control and analgesia (Albuquerque et al., 2009).

nAChRs are always pentamers, composed of five different subunits: alpha (α1-α10), beta (β2-β5), delta, epsilon and gamma whose combination differs in different tissues. The α subunit contain the recognition site for ACh, the β subunit does not bind ACh. However, it is important for the interaction between the ligand and α subunit. Neuronal nAChRs are heterogous or homogenous pentamers composed of either combination of α and β subunits or by five alpha7 subunits, respectively (reviewed in Arias et al., 2006).

4.4 Cholinergic system in the striatum

Cholinergic system in the striatum is mainly represented by intrinsic CINs, which make rich connections to MSNs. CINs exhibit spontaneous firing rate and based on this, they are also sometimes referred to as tonically active neurons (TANs).

Another source of acetylcholine in striatum are projections from the PPT/LDT complex.

Acetylcholine receptors expressed in striatum are mainly M1 (excitatory) and M4 (inhibitory) expressed by MSNs. M2 and M4 autoreceptors are also expressed by CINs. In addition, both inhibitory M2 mAChRs and excitatory nAChRs are expressed by dopaminergic and glutamatergic terminals in the striatum (Hersch et al., 1994; Quik and Wonnacott, 2011).

4.4.1 Morphology of striatal CINs

CINs were first described by Kolliker in his studies of Golgi stained material in the late 1800's (Kolliker, 1896). Golgi staining and intracellular labelling with immunocytochemistry for ChAT elucidated that Kolliker's interneurons are cholinergic cells (Kawaguchi, 1993). Because of extensive arborizations, striatum has the highest expression of ACh and ChAT (Woolf et al., 1984). There is also significant number of varicosities which are axonal enlargements containing aggregated synaptic vesicles on these interneurons. Quantitative electron microscopy indicates that there are about $2*10^8$ ACh varicosities/mm^3 in the striatum, and each striatal cholinergic interneuron has 500,000 axon varicosities. A 10 µm-radius sphere of striatal neuropil contains about 400 DA and 400 ACh axon terminals (Descarries and Mechawar, 2000).

Interestingly, there is difference in morphology between CINs in the dorsal and ventral striatum. In primates, the ventral striatum contains CINs with round or elongated cell bodies, as well as sparsely branched dendritic trees, while CINs in the dorsal portion of the striatum display large cell bodies with thick primary dendrites, the "spider-like" dendritic trees and widespread intrastriatal axonal arborizations (Gonzales and Smith, 2015).

4.4.2 Physiological properties of CINs

Cholinergic interneurons are very important regulators of striatal connectivity and output. Electrophysiologically, they are characterised as TANs, which can produce spontaneous tonic firing without activation by synaptic input. Striatal TANs in rodents and monkeys exhibit a large depolarized membrane potential (approximately –60 mV), tonic spike discharge around 2–10 spikes/s and broad spike waveforms. They can also exhibit various spiking patterns (i.e. regular, irregular, and bursting) (Wilson et al., 1990a).

4.4.2.1 Tonic firing in CINs

Tonic firing is generated by intrinsic membrane properties by activation of sodium $Na_V1.6$ channels at subthreshold voltage. A sodium current then depolarizes the cell membrane, which causes opening of calcium voltage activated channels $Ca_v2.1$, which leads to action potential. Calcium current then activates calcium- (A-type) and voltage-dependent potassium channels (both BK and SK), which produce medium-duration after-hyperpolarization (mAHP). Finally, the hyperpolarization activates the depolarizing current $I_{h,}$ mediated by cationic HCN channels. This provides an inward ion current that activates the persistent Na^+ current and brings back the voltage to the point where subthreshold sodium channels are activated. Then the threshold is reached and the cycle repeats (Bennett et al., 2000).

4.4.2.2 Pause response in CINs

In awake monkeys exposed to repeated, motivationally significant stimuli, it was observed that CINs respond by a pause in firing (Kimura et al., 1984), sometimes preceded and often followed by a burst. This pause is associated with environmentally salient stimuli, which are followed by a reward or punishment, which is correlate to classical conditioning, thus it is sometimes referred to as conditioned pause response.

The mechanism underlying the generation of the pause has been investigated for years but still is not completely understood. Principally, the pause can be caused by two mechanisms, either

by direct synaptic inhibitory input or by suppression of spikes due to intrinsic cellular mechanisms.

Cholinergic interneurons can be inhibited GABA input which inhibits their activity through the mechanism involving NPY-expressing neurogliaform interneurons and probably another type of GABAergic interneurons. Activation of these GABAergic interneurons depends on ionotropic nAChRs receptors rather than G-protein coupled mAChRs receptors (English et al., 2012).

Another possible source of the pause could be short membrane potential depolarizations. Intrinsic membrane mechanisms of cholinergic interneurons can cause AHP after action potential. Ionic currents that are involved in generation of the pauses are probably caused by activation of a slow AHP-type calcium-activated potassium channels (I_{sAHP}) and transient deactivation of the hyperpolarization and cyclic nucleotide-activated cation channels (I_H). This usually causes tonic depolarizing drive which can also cause a subsequent pause in firing activity of CINs (Goldberg and Wilson, 2005; Oswald et al., 2009; Wilson et al., 1990b).

In addition, excitatory input from the thalamus is probably influencing pause response in CINs. In primates, it has been shown that injection of muscimol, a $GABA_A$ receptors agonist, into the centromedian–parafascicular complex of the thalamus significantly reduced the pause response of CINs to a stimulus that had previously been associated with a reward (Matsumoto et al., 2001). In rodents, stimulation of thalamic afferents can evoke excitation – pause responses in cholinergic interneurons, with the pause in vivo exhibiting the characteristics of an AHP (Goldberg and Wilson, 2005; Schulz et al., 2011).

Finally, it is also thought that dopamine has direct effect on pause in CINs. Dopamine acts through inhibitory D2 receptors expressed by CINs, which can hyperpolarize the CINs' membrane. In non-human primates, spike bursts in dopaminergic neurons projecting to the striatum are nearly coincident with the pauses in CINs, revealing that they may be related. However, CINs do not show the same reward sensitivity as dopamine neurons, suggesting that dopamine is not probably involved directly in generating pauses but can be important modulator of pause characteristics through membrane hyperpolarization (Joshua et al., 2008; Morris et al., 2004).

4.4.3 Afferent connections to CINs

4.4.3.1 Glutamatergic input

Glutamatergic input comes mainly from the cortex and thalamus. Cortical projections to CINs have origin in various regions of the cortex. These regions include mainly motor cortex, somatosensory complex, secondary visual cortex and cingulate cortex.

The lateral and medial parafascicular thalamic nuclei in rodents, which are homologous to centromedian/parafascicular (CM/Pf) complex of thalamus in the primates, are major excitatory glutamatergic input to the striatum including CINs (Lapper and Bolam, 1992). Glutamatergic inputs are likely responsible for synchronous activation of CINs, which probably coordinate DA release through activation of nAChRs on DA terminals (Threlfell et al., 2012). Glutamate induces depolarization through activation of postsynaptically expressed AMPA, NMDA, and kainate receptors.

4.4.3.2 Dopaminergic input

Dopaminergic input to striatal CINs comes mainly from the SNc. Majority of striatal cholinergic interneurons express D2 with highest density in the dorsolateral caudate – putamen, and D5 receptors both in ventral and dorsal striatum. Notably, D1 receptors has been found on the CINs in ventral but not dorsal striatum in primates (Nicola et al., 2000). D2 receptors generally suppress neuronal excitability by activation of Gi proteins, which inhibits formation of cAMP. In contrast, D5 receptors increase neuronal excitability by activation of Gs proteins, which stimulate formation of cAMP (Grandy et al., 1991; Olianas and Onali, 1992).

4.4.3.3 GABAergic input

Cholinergic interneurons receive GABAergic input mainly from striatal sources. GABA can inhibit cells by activating ionotropic $GABA_A$ receptors, which increases Cl-conductance. GABA can also activate metabotropic $GABA_B$ receptors, which are G-protein coupled and decrease cell excitation by coupling to the $G_{i/o}$ protein as well as negatively regulate adenylylcyclase (AC).

GABAergic MSNs provide major input to striatal interneurons in the putamen in primates. Neurons that express D1-type dopamine receptors make richer connections to CINs than neurons with D2-type dopamine receptors, thus D1 MSNs has greater influence on CINs (Martone et al., 1992). In addition, striatal GABAergic interneurons also form inhibitory synapses with CINs (English et al., 2012).

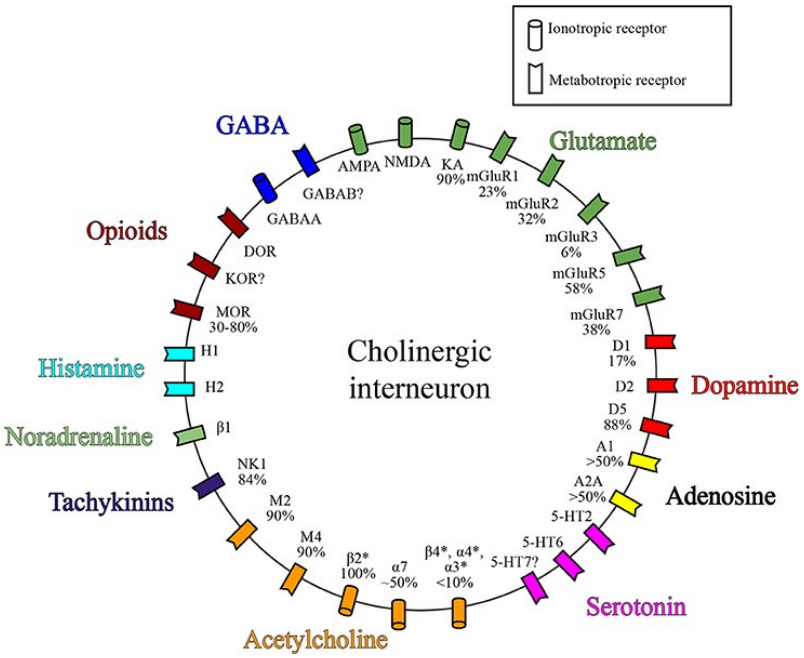

Figure 3: Receptor expression on ChIs, adopted from Lim 2014

4.4.4 Efferent connection of CINs

CINs make large arborization and thus plentiful connections across the striatum. They modulate striatal microcircuits through various types of nAChRs and mAChRs expressed by striatal neurons and terminals.

nAChRs are expressed both pre and postsynaptically. Presynaptic nAChRs enhance release of neurotransmitters by rapid depolarization and postsynaptic nAChRs cause depolarization and increase excitability.

Mainly M1, M2 and M4 subtypes of mAChRs are expressed in the striatum, by MSNs and on projection terminals as well.

4.4.4.1 Medium spiny neurons

CINs have rich dendritic and axonal connections that integrate most of striatal synaptic inputs as well as its output via MSNs. CINs control striatal projection neurons mainly via mAChRs and nAChRs (Bolam et al., 1988). Interestingly, nAChRs are not expressed by MSNs or only in very low levels (Matsubayashi et al., 2001). mAChRs on MSNs are predominantly M1, which increase excitation, and M4, which decrease the membrane potential, thus have inhibitory effect on excitability. Both types are expressed differentially in MSNs. MSNs involved in the direct pathway (D1-MSNs) express both M1 and M4 mAChRs, while indirect pathway MSNs (D2-MSNs) express predominantly M1.

4.4.4.2 GABAergic interneurons

Another way how CINs control the striatal output is by the indirect inhibition of MSNs through GABAergic interneurons which then project to MSNs. This disynaptic connection consists of nAChRs expressed GABAergic interneurons activated by ACh and these interneurons then release GABA onto MSNs (English et al., 2012).

In addition, CINs can act on GABA interneurons by activation of muscarinic receptors. Expression of the M2 receptor has been demonstrated in the NPY+ and PLTS interneurons (Bernard et al., 1998). ACh can decrease GABA release from interneurons by its effect on inhibitory mAChRs and thus decrease the GABA action onto MSNs (Lim et al., 2014).

4.4.4.3 Glutamatergic terminals

Glutamatergic input in the dorsal striatum comes primarily from the intralaminar nuclei of the thalamus and from the sensorimotor cortex, with a small amount of glutamate co-released from other terminals as well (Higley et al., 2011). CINs are important regulators of excitatory inputs to MSN, both rapidly by acting on nAChRs, and more slowly and persistently via mAChR activation

Activation of nAChR expressed by glutamatergic terminals in the striatum increases glutamate release by increased $Ca2^{+}$ influx, therefore the nAChRs directly enhance neurotransmitter release (Campos et al., 2010).

ACh binding on mAChRs has negative effect on striatal glutamate release. Modulation of glutamatergic terminals is realized through activation of presynaptic M2 and M4 type of muscarinic receptors (Hersch et al., 1994; Levey et al., 1991).

4.4.4.4 Dopaminergic terminals

Cholinergic interneurons synapse onto DA terminals, regulating their transmitter release by acting on nAChRs expressed by DA terminals. Activation of the nAChRs enhances DA transmission. DA terminals express α4 and β2 subunits, α5, α6, α7, and β3 subunits at various levels (Grady et al., 2007; Le Novere, 1996; Sharples et al., 2000).

It has been measured that nicotinic agonists increase the efflux of DA in striatal tissue, (Campos et al., 2010) and nAChR antagonists decrease DA release through the action on presynaptic nAChRs on DA terminals (Wonnacott et al., 2000). Thus, CINs are strong regulators of

dopamine release. It has been shown that nicotinic activation is sufficient to drive dopamine release in striatum even without the firing activity of the midbrain dopaminergic neurons projecting to the striatum (Threlfell et al., 2012).

5 Cognitive flexibility

Cognitive flexibility is described as the ability to switch between different mental sets, tasks or strategies in order to adapt to changes in the environment. It belongs to executive functions such as working memory, attention control, cognitive inhibition and inhibitory control. Generally, we can distinguish three types of tasks commonly used to probe cognitive flexibility, including reversal learning, intra-dimensional set shifting and extra-dimensional set shifting.

Impaired or reduced cognitive flexibility can be found in various neuropathological conditions such as obsessive-compulsive disorder (OCD), autism, addiction, Parkinson's disease, schizophrenia, eating disorders etc. For example, patients with OCD have difficulties with shifting between mental processes to generate adaptive behavioural responses. Similarly, deficits in cognitive executive functions, such as attentional set shifting and task switching are present in patients with schizophrenia (Kehagia et al., 2010).

5.1 Methods of measuring cognitive flexibility

Although different approaches are used for testing cognitive flexibility in humans and mouse models, basic principles are the same because they measure similar behavioural functions.

At the beginning, a subject is introduced to a new environment with new stimuli, which are relevant to a task, which need to be solved in order to receive a reward. In the initial stage, responses to relevant stimuli are strengthen by conditioning and response to other stimuli are weakened. After the subject is learned to a specific task, reinforcement is shifted to a new task, which was not previously rewarded, and the old task is not followed by a reward anymore. Subject then finds out that previous strategy is not working and must inhibit previous strategy and learn a new strategy that leads to a reward. Below are briefly discussed the most commonly used cognitive flexibility test for humans and several tests used in mice.

5.1.1 Wisconsin Card Sorting Test (WCST)

WCST is commonly used neuropsychological test for testing set shifting and it is one of the most frequently used executive functions measures. In this test participant is told to sort number

of cards to match either colour (red, blue, yellow or green), form (crosses, circles, triangles or stars) or number of figures (one, two, three, four), however, the participant is not explicitly told what rule to follow. During the task, he or she receives an immediate feedback, that is, he/she is told whether a particular match is right or wrong. During the task the sorting rule can be changed any time, thus, the participants have to shift their strategy accordingly and sort card following the actual rules.

5.1.2 Intra-dimensional/extra-dimensional digging task

This assay is a rodent analogue of the WCST. Rats (Birrell and Brown, 2000) or mice (Bissonette et al., 2008) have to dig in sand in the correct location that can be determined based on the dimension of texture or smell. The rule (follow either smell or a texture) is again changed at some point during the task.

5.1.3 Spatial reversal in Morris water maze and T-maze task

The Morris water maze is one of the most common rodent tests for learning and memory. Rodents are placed in a large circular pool of water where they locate a submerged platform. Visual cues are placed around the pool so the animal can relate the environment with the location of platform. To probe cognitive flexibility, in next set of trials, the location of platform is changed and the ability of extinguish their initial learning and acquire a new path to the new position of platform is tested (Morris, 1984).

T-maze is a closed apparatus in a form of T with base and two goal arms, one of them containing a reward. Mouse has to learn to choose one arm to get the food reward and the location of the correct arm is changed in order to test the cognitive flexibility (Deacon and Rawlins, 2006).

5.1.4 Reversal learning tested in operant boxes

Operant box is an apparatus with operandum which is usually a lever which can be pressed by animal. Operant box is usually soundproof and lightproof in order to avoid distracting stimuli. In a bar-pressing system, the mouse learns that after pressing a correct lever, that is the one associated with light (visual cue) or location (only left/right), gets rewarded. Cue is then switched, and animal is tested for reversal learning. In a touch screen-based operant system animal is learned to discriminate and reverse between visual stimuli which are projected on a touch screen. This assay is more comparable to reversal task employed in human testing.

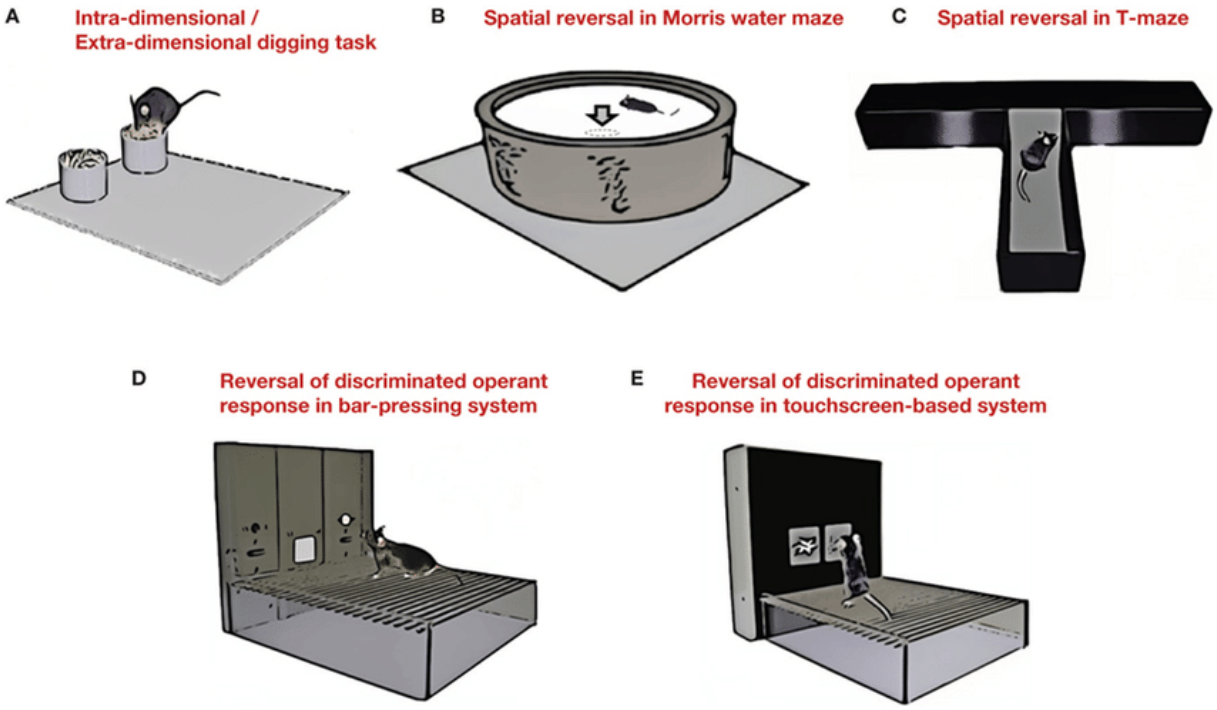

Figure 4: Mouse bahavioural assays for cognitive flexibility, adopted from Brigmann et al 2010

5.2 Neural structures underlying cognitive flexibility

Investigations into neurological correlates of cognitive flexibility have revealed a variety of frontoparietal brain regions involved in flexible switching. These regions include prefrontal cortex, anterior cingulate cortex and posterior parietal cortex. Notably, among the subcortical regions involved in the control of cognitive flexibility are basal ganglia and the dorsomedial striatum in particular.

5.2.1 Prefrontal cortex

Prefrontal cortex covers rostral part of the frontal lobe, it plays an essential role in organisation and the control of goal directed behaviour. Prefrontal cortex is commonly divided into medial prefrontal cortex, which can further be subdivided into anterior cingulate (Ac), infralimbic (IL), and prelimibic (PrL) subregions. Similarly, orbitofrontal cortex (OFC) is usually subdivided into medial (MO), ventral (VO) and lateral (LO).

The orbitofrontal cortex is involved in decoding some primary reinforcers such as taste; in learning and reversing associations of visual and other stimuli to these primary reinforcers; and it plays an executive function in controlling and correcting reward-related and punishment-related behaviour, and thus in emotion.

Medial prefrontal cortex along with anterior cingulate plays role in more complex functions of cognitive flexibility. These include: (1) forming associations between stimuli, responses and outcomes, (2) detection of errors and conflict between rules, (3) tracking of reward history to determine which responses are no longer valid, and (4) enhanced attentional processes to resolve these issues when rules are violated (Bissonette et al., 2013).

5.2.2 Striatum

For morphological descriptions, see chapter 1. Striatum plays a key role in planning and coordination of motivated movements, in executive functions such as visual working memory (Voytek and Knight, 2010), cognitive flexibility (Floresco et al., 2009) and reward-based decisions (Morris et al. 2004). It has been shown that inactivation of the dorsomedial striatum affects acquisition, reversal learning, and extradimensional shifts of different discrimination tests. This striatal region is crucial for maintenance and execution of previously relevant strategy and for the generation of a new strategy, but it is not critical for the initial inhibition of the previously relevant strategy (Ragozzino, 2007).

Nucleus accumbens, which is a ventral part of the striatum, plays an important role in processes associated with motivation and reward. It consists of core and shell, which both has rich connectivity with PFC. In experimental inactivation of nucleus accumbens core, animals were unable to shift from egocentric to allocentric strategy and thus they were unable to learn the new strategy. Inactivation of nucleus accumbens shell led to faster set shifting. This is probably because the shell is responsible for distinguishing between important and unimportant stimuli, which are then ignored, but with inactivation of shell it's easier for an animal to choose a new strategy (Brog et al., 1993).

5.2.3 Mediodorsal thalamus

Thalamus is a mass of grey matter, which is traditionally, divided into many nuclei that projects reciprocally to the cortex. This thalamo-cortical system is involved in large scale neural dynamics (Schmitt et al., 2017).

Mediodorsal thalamus (MD) projects reciprocally to the prefrontal cortex including orbitofrontal and prelimbic cortices, it has also rich projections to the core of the NAc (Berendse and Groenewegen, 1990). Inactivation of MD in experiments with rats showed disrupted shifting from response to visual cue discrimination strategy, and vice versa. Asymmetrical disconnection by lesioning of the MD in one hemisphere and the PFC in the other also caused a perseverative deficit when rats were required to shift from a response to a visual cue

discrimination strategy, as did disconnections between the PFC and the NAc. However, inactivation of the MD on one side of the brain and the NAc contralaterally resulted in a selective increase in never-reinforced errors, suggesting this pathway is important for eliminating inappropriate strategies during set shifting (Block et al., 2007).

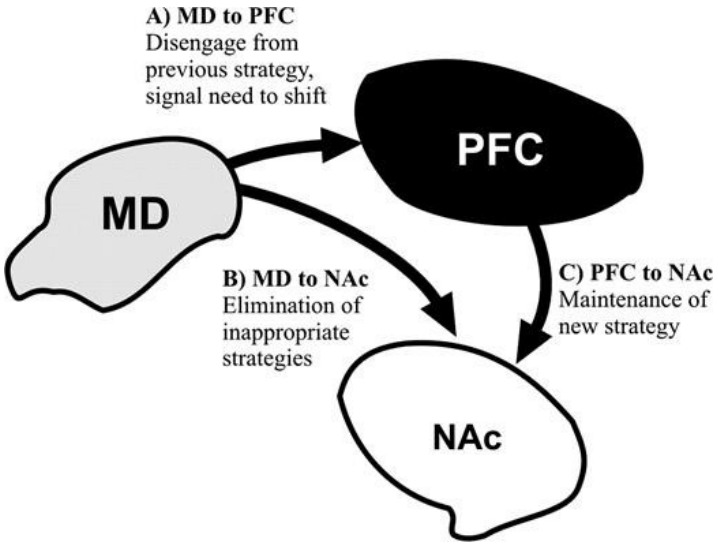

Figure 5: Schematic of main neural structures involved in cognitive flexibility, adopted from Block et al 2007

6 Cholinergic signalling in the striatum and cognitive flexibility

6.1 CINs are important for the detection of salient cues

The firing activity of striatal CINs reflects occurrence of environmentally and behaviourally relevant stimuli. Specifically, it has been shown in primates (*Macaca fascicularis*) that CINs (in many studies referred to as TANs based on their electrophysiological properties) respond with short-lasting depression or activation in their otherwise tonic activity to stimuli that were relevant for performing the behavioural task. In the seminal study by (Apicella et al., 1991), the authors used microelectrodes placed in the left striatum of two monkeys during contralateral performance of delayed go-nogo task. Among those TANs whose activity was phasically modulated were 85 %, which responded with initial depressions, and 14 %, which responded with initial activation. Additionally, depressions were usually followed by a rebound activation. The responsiveness to novel and/or unexpected stimuli might be mediating the TANs' ability to respond to changes in contingencies and enable the animal to adapt its behavioural strategy when changes in the environment occur.

6.2 Early microdialysis and pharmacological studies

Since Apicella's study, other studies followed suggesting that the activation of CINs in the DMS play a significant role in the detection of changes of contingencies and cognitive flexibility. Early studies come from the group of Michael Ragozzino; this research group examined influence of CINs in behavioural tests performed by rats in the reversal variant of Morris Water Maze. They also used microdialysis and intrastriatal cannulation to measure ACh levels and deliver ligands of individual ACh receptors to establish their role. In (Ragozzino and Choi, 2004), the authors measured ACh levels *in vivo* with microdialysis in the dorsomedial striatum during the reversal learning phase of the place discrimination task. The task was performed in a modified cross maze where rats learn to enter one arm to receive a reward (acquisition) and on the second day (reversal learning) of testing, the rats learn to enter the opposite arm that was not reinforced during acquisition. There was no increase of ACh output during the acquisition phase of the task but significant increase of ACh during reversal learning. In addition, as rats were improving their performance in a newly learned strategy, ACh levels were gradually decreasing again (Ragozzino and Choi, 2004).

Moreover, in other studies, the Ragozzino's group used non-specific lesions or infusions of local anaesthetics showing effects that further suggest involvement of DMS in cognitive flexibility. Infusion of the local anaesthetic tetracaine into DMS impaired strategy shift in response/place discrimination learning. In the T-maze, rats had to switch from a response strategy (left or right body turn) to a visual cue. Here, tetracaine treated rats took longer to meet reversal learnig criteria comparing to the control group. When the rats' performance was examined more closely, there was a specific increase in a number of regressive errors, while the perseverative errors did not differ from controls. This means that the rats with inactivation of DMS did not have any difficulties to stop using the previously learned response, but in the later stages of the reversal task they more often reverted to previously learned, now in-correct, response (Ragozzino et al., 2002a). These two types of errors, perseverative and regressive, are commonly used as a parameter of performance in the reversal learning tasks. The perseverative error reflects the inability to shift away from the previously correct response and it is calculated as errors made in the early stages of the reversal learning task, when animals perform with less than 50 % accuracy. In contrast, the regressive error reflects errors, which are made after the rat stops perseverating, and is calculated as errors made in later sessions when animals reach at least 50 % accuracy.

In a similar study, also the role of activation of CINs through NMDA receptors was investigated. The authors hypothesized that when a shift of strategies is needed during the reversal learning task, the activation of NMDA receptors is necessary to increase the acetylcholine efflux in DMS. Accordingly, mice with blocked NMDA receptors with DL-2-amino-5-phosphonopentanoic acid (AP-5) showed decrease of ACh output during the task as well as impairment of the reversal learning (Palencia and Ragozzino, 2006). Again, this suggest ACh signalling in striatum plays an important role in modulating reversal learning.

In pharmacological studies, more or less specific antagonists of individual subtypes of mAChRs were used to assess their functions in cognitive flexibility tasks. It is important to note that relatively non-specific ligands of individual muscarinic receptors have been used in some experiments. Hence, depending on the local concentration, the used drug might have affected also other receptor subtypes different from the primary intended target. Therefore, the conclusions of the pharmacological studies on the function of individual subtypes of mAChRs have to be taken with caution and with keeping in mind the specific drug, its actual specificity and the concentration that was used in the study. That being said, bilateral infusion of M1 mAChR antagonists (muscarinic toxin 7, pirenzepine) and non-specific muscarinic antagonist (scopolamine) had no effect on acquisition phase of learning but had negative effect on reversal learning in place discrimination task, which led to slower learning of a new strategy. Infusion of muscarinic toxin 3 (MT - 3) which is an antagonist of the M4 mAChR had no effect on place acquisition or reversal learning (McCool et al., 2008; Ragozzino et al., 2002b). Finally, bilateral injections of oxotremorine sesquifumurate, which is an agonist of M2 mAChR, into DMS reduced cholinergic transmission; this impaired reversal learning and diminished the increase of ACh release. When oxotremorine was injected with AF-DX-116, a M2 receptor antagonist, it had the opposite effect, the ACh levels and the behavioural deficit caused by oxotremorine were reversed and were comparable to controls (Ragozzino et al., 2009).

According to the studies described above, different subtypes of mAChRs may differ in their effect on cognitive flexibility. The data are suggesting that M1 and M2 receptors may play an opposing role in modifying cognitive flexibility while M4 does not seem to play any role.

6.3 Importance of thalamic input to CINs for the control of cognitive flexibility

The CINs are receiving excitatory thalamic input that seems to be even more functionally relevant for them than the cortical input (Lapper and Bolam, 1992). Several studies have indicated that the activation by the thalamic input is necessary for proper functioning of CINs

in the control of cognitive flexibility. Specifically, removal of the excitatory input from the parafascicular (Pf) nucleus of the thalamus to the DMS led to the reduction of behaviourally induced increase of ACh levels in the DMS. This was associated with an impairment of cognitive flexibility in the place discrimination task (Brown et al., 2010) and in the contingency reversal task (Bradfield et al., 2013). Specifically, in the place discrimination task, rats with the Pf lesion had more difficulties than controls to choose the new correct arm in the T-maze. Similarly, the rats in the contingency reversal task had lower ability to learn to switch a new lever in the operant box after the association with the reward changed. Notably, unilateral Pf inactivation did not impair reversal learning, only when Pf was bilaterally inactivated. Additionally, this treatment had no effect on initial acquisition of the task (Brown et al., 2010). Surgically lesioned thalamostriatal tract leading from Pf to posterior DMS, which caused chronical elimination of Pf input to CINs on one side and pharmacologically blocked cholinergic activity in DMS contralaterally was tested in the following research. The rationale for this experiment are that if these two regions are functionally connected, then combination of unilateral lesion of Pf with an unilateral lesion of DMS in the contralateral hemisphere should disrupt their function. In this experiment, the rats were tested in the contingency degradation in which outcome is delivered outside of the lever press-outcome contingency in such a way that probability of the outcome is the same whether the animal presses the lever or not. In addition to the contingency degradation, rats were tested in the outcome devaluation, where animals were given unrestricted access to either pellets or sucrose followed by a choice extinction, testing if the rats will press the lever which paired outcome had not been previously devalued. Results of these tests confirmed that Pf lesioned rats are unable to use action-outcome information to guide instrumental performance when the initial contingencies were changed. These cytotoxic lesions of the Pf had no effect on initial goal-directed learning but impaired the ability of rats to adjust to change in the action-outcome contingency. Consequent immunoassays confirmed effect on CINs that had decreased activity and increased activity of MSNs, which was possibly due to the loss of the general inhibitory effect of CINs on MSNs (Bradfield et al., 2013). Data from these studies suggest that CINs' activity is necessary to reduce interference between learning the original and the new contingencies. They further suggest that the thalamostriatal pathway exhibits state control over learning-related plasticity in the DMS (Bradfield et al., 2013; Brown et al., 2010). In agreement with these findings, in (Matamales et al., 2016) genetic ablation of CINs in the posterior DMS and cholinergic inactivation by parafascicular-thalamic modulation was accompanied by impairment in place reversal learning and by impaired adaptation to changed contingency in operant chambers. This

study also demonstrated that aged mice display deteriorated Pf thalamic function, which leads to reduced activity of CINs in DMS and to impaired ability to encode new learning.

6.4 Specific lesions and chemogenetic inhibition of striatal CINs

In addition to these studies, some groups used immunotoxin anti-ChAT IgG-saporin to make specific lesions of CINs in the DMS or VS of rats (Ragozzino et al., 2002a; Aoki et al., 2015). These lesions had no significant effect on initial learning. Moreover, the ablated CINs in the VS did not affect the reversal learning. In (Aoki et al., 2015), only the animals with ablation of CINs in the DMS showed impaired set shifting which was expressed as an increase in perseverative errors. This is in contradiction to (Ragozzino et al., 2002a), where impairment was observed as an increase in regressive errors. Overall, these data suggest that DMS but not VS CINs play a role in inhibiting the previous strategy (Aoki et al., 2015).

Investigating the role of DLS CINs in behavioural flexibility, chemogenetic activation of CINs with hM3Dq Designer Receptors Exclusively Activated by Designer Drugs (DREADD) increased their firing rate and occluded the typical pauses (Aoki et al., 2018). The chemogenetically treated mice were tested in habit substitution task in the operant box where they increased their response rate represented by shorter inter-press intervals. According to authors, this suggests involvement of DLS CINs in modulating the expression of a new habit response. Taken together, these studies suggest that along the DMS, also DLS play its role in the control of cognitive flexibility, while there is still a need of further research to elucidate their exact function (Aoki et al., 2018).

Ventromedial striatal TANs (putative CINs, electrophysiologically identified) activity has been described in (Atallah et al., 2014). Recordings showed firing modulation in TANs in response to unexpected reward (increase of the firing rate) and omission (decrease of the firing rate) when performing a reward-based learning task.

6.5 Studies showing negative or no effect of CINs on cognitive flexibility

Interestingly, in direct contrast to studies listed above, in (Okada et al., 2014) authors found that selective elimination of DMS CINs enhances reversal learning and extinction of a place discrimination task performed with long inter-trial intervals (ITI) of 20 minutes. In (Okada et al., 2018), the same research group examined the impact of CINs ablation on performance in response discrimination task with reversal phase using three different ITIs. Their results

demonstrate that DMS CINs mediate inhibition in both place and response reversal performance with a relatively longer ITI, but their functions differ between types of reversal performance in the tasks with a shorter ITI. Specifically, elimination of DMS CINs resulted in impaired reversal performance of response discrimination with the short ITI schedule (15 s). With the middle ITI (10 min), rats performed better in response discrimination, similar results were observed with the long ITI (20 min) where rats again performed better in reversal performance of response discrimination. In none of these tests using different ITI, the CINs ablation had influence on initial acquisition. Therefore, these findings suggest that the influence of striatal CINs ablation on reversal performance with different ITI schedules is probably associated with the contribution of CINs to the time-dependent plasticity changes, which are crucial for learning and memory processes in the striatum. In other words, CINs in the DMS play distinct roles in behavioural flexibility dependent on the trial spacing and discrimination type constituting the learning. Thus, trial spacing may be important in explaining the inconsistency among some studies described above (Okada et al., 2018).

6.6 Human data

In addition to animal studies described above, methods of proton magnetic resonance spectroscopy were used to measure levels of choline in human subjects. It was found that levels of choline in the dorsal striatum were associated with reversal learning, but not with initial learning performance (Bell et al., 2019). This only supports the notion that what was found in the animal studies discussed above also applies to human subjects. With further development of non-invasive methods for measuring the activity of CINs in human subject, we can expect more studies in the future, which will bring more knowledge about striatal cholinergic signalling. It will be interesting to see if this will confirm the results from animal studies.

6.7 The effect of CINs on cognitive flexibility may have therapeutic implications

In relation to human pathologies, lesions of striatal CINs have been associated with social deficits and stereotypic behaviour (Rapanelli et al., 2017) and they were found to worsen ritualistic-like behaviours that affect social performance (Martos et al., 2017). These signs of social impairment and stereotypic behaviour are common in some neuropsychiatric disorders such as autism or obsessive-compulsive disorder. In addition, impairment of cognitive flexibility has been found in other human pathologies often associated with disturbances in (cortico-)striatal circuits including Parkinson's disease, schizophrenia and eating disorders (D'Cruz et al., 2013; Karvat and Kimchi, 2014; Miller et al., 2015). We can conclude that all

studies discussed in this chapter confirmed strong influence of striatal CINs in modulation of cognitive flexibility. We can expect continuous research in striatal cholinergic signalling and further development of substances targeting cholinergic receptors as a cure for these psychiatric disorders.

7 Conclusion

CINs are a major source of ACh in the striatum and they play many important roles in the modulation of striatal functions. Distribution of ACh receptors throughout the striatum together with characteristics of CINs firing implies their specific role in the modulation of the striatal activity and output. Inputs from the cortex and thalamus to these neurons may also indicate their involvement in behavioural processes. Considering studies described above, there is no doubt that CINs' activity has an impact on cognitive flexibility. Although during the past 20 years CINs were undergoing extensive research, there are still questions about their functions. For example, co-release of other neurotransmitters such as glutamate and GABA (Gras et al., 2008; Lozovaya et al., 2018) by these neurons has been documented. Therefore, pharmacological blockage or lesions of CINs would not impair only ACh release but other co-transmitters as well. Methods of genetic engineering like genetically modified mice in which specific genes for proteins involved in cholinergic synthesis and transmission are knocked out, optogenetics, where activity of neurons can be controlled using optical instruments or chemogenetics, where engineered macromolecules are used to control the neuronal activity, could shed more light on how these cholinergic neurons influence striatal functions. These approaches may reveal how CINs function is distorted in disease states affecting the basal ganglia and provide new therapeutics for disorders involving the basal ganglia circuits.

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